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(21) International Application Number: PCT/US99/28014 (22) International Filing Date: 23 November 1999 (23.11.99) (30) Priority Data: 09/199,655 25 November 1998 (25.11.98) US (71) Applicant: AFFYMETRIX, INC. [US/US]; 3380 Central Expressway, Santa Clara, CA 95051 (US). (72) Inventors: MATHIES, Richard, A.; University of California, Berkeley, Dept. of Chemistry, Berkeley, CA 94720 (US). SIMPSON, Peter, C.; University of California, Berkeley, Dept. of Chemistry, Berkeley, CA 94720 (US). (74) Agents: ZAITLEN, Richard, H. et al.; Pillsbury Madison & Sutro LLP, Suite 1200, 725 S. Figueroa Street, Los Angeles, CA 90017 (US).		(81) Designated States: JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: MICROPLATE SAMPLE AND REAGENT LOADING SYSTEM (57) Abstract Apparatus for forward and reverse transfer of fluids through capillaries, consisting of at least one capillary, a pressure generating or housing means, which preferably includes a box, having first and second means for aligning the capillaries from one set of wells to a second set of wells, and applied pressure differential transfers small amounts of liquid uniformly and in parallel. A method of accurately controlling a desired volume of fluid flow is particularly useful for transferring liquids to and from a microtiter dish to a Capillary Array Electrophoresis Microplate having liquid wells spaced in a radially symmetric configuration, or for maximizing desired transfer of the like or improved novel enhanced patterned arrays.		

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MICROPLATE SAMPLE AND REAGENT LOADING SYSTEM

Background of the Invention

Research leading to portions of the present invention was funded in part by the
5 National Institutes of Health and by the Department of Commerce through the
National Institute of Standards and Technology.

Area of the Art

The present invention relates to methods and apparatus useful for small
volume liquid transfer. In particular, the present invention relates to facilitating both
10 forward and reverse parallel liquid transfer of aliquots of solutions from at least one
reservoir to a different reservoir, as is especially useful in the context of systems for
electrophoretic analysis, such as with Capillary Array Electrophoresis ("CAE")
Microplates.

Description of the Prior Art

15 Prominent among the conventional methods and apparatus for the transfer of
liquids are robotic and like automated systems. However, owing to cost and the lack
of flexibility of such systems, numerous drawbacks have arisen. Likewise, the trend
toward automating and enhancing the efficiency of DNA mapping and sequencing
technology has pushed the envelope of several related fields of art which have been
20 synthesized serendipitously by the present inventors to generate the unexpected results
of the present invention.

By way of background, means for the detection of samples within capillary
tubes using methods such as confocal microscopy are disclosed by United States
Letters Patent No. 5,091,652 ("Mathies et al.") which issued on Feb. 25, 1992, to one
25 of the present inventors. United States Letters Patent No. 5,560,811 ("Briggs et al.")
issued Oct. 1, 1996 and is assigned to Seurat Analytical Systems, Inc. The subject
matter is a method and apparatus for multiplexing electrophoresis analysis. Briggs et
al. offers for consideration an excellent summary of the evolution of the instant
technology and a thorough description of the state of the art.

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United States Letters Patent No. 5,443,791 ("Cathcart et al."), issued Aug. 22, 1995 and assigned to the Applied Biosystems Division of Perkin Elmer, discloses an "Automated Molecular Biology Laboratory." This system employs an expensive and complex robotic translation mechanism. United States Letters Patent No. 5,770,157 ("Cargill et al.") for "Methods and Apparatus for the Generation of Chemical Libraries" likewise focuses on costly and time-intensive facilitation of robotic manipulation. United States Letters Patent No. 5,540,888 ("Bunce et al."), issued July 30, 1996 to the British Technology Group, Ltd., for "Liquid Transfer Assay Devices," is further representative of the state of the art. The Bunce et al. device requires first, second, third, and fourth flow channels of porous material. "Application Specific Capillary Electrophoresis" was disclosed by United States Letters Patent No. 5,372,695 ("Demorest"), held by Applied Biosystems, Inc., which issued on Dec. 13, 1994. This system included a complex serving apparatus which impeded its commercialization.

Users of liquid transfer systems have been dissatisfied with the systems described by the above patents. These users continue to demand greater flexibility and cost efficiency. According to the present invention, there is no need for an expensive serving apparatus, owing to the invention's speed and efficiency. One may moreover use any number of capillaries, and accurately dispense extremely small submicroliter volumes. These teachings embrace and finally address the clear need for a liquid transfer system which is operational at high speeds and is more effective than conventional systems.

Summary of the Invention

A microplate sample and reagent loading system transfers small μl or sub- μl volumes of liquid from one liquid-containing well to a second liquid-containing well. Alternatively, the system may be used to transfer μl and sub- μl volumes from a first array of liquid-containing wells to a second array of liquid-containing wells. A first end of an array of capillaries is placed into a solution in a first set of wells located inside of a pressurized chamber. A second end of the array of capillaries is arranged

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by a second manifold into a configuration corresponding to a second set of reservoirs. By the application of a predetermined amount of pressure for a predetermined amount of time, a small aliquot of liquid is transferred through each capillary in the array, performing uniform transfer of a plurality of solutions in parallel.

5 The volume of the transferred solution is controlled by applying a controlled pressure and by precisely defining the time that the pressure is applied. Alternatively, the transfer could be driven by placing a second (or third) set of reservoirs in a second (or third, etc...) chamber and transfer effected by applying a vacuum to each respective chamber. Likewise, either forward or reverse vacuum pressure can be
10 applied to the first pressure box to draw solutions into the wells which are contained therein.

 Capture of a desired solution is effected, according to an embodiment of the instant teachings, by controlling the flow and fixing the same in a specific location by, for example, freezing a small plug of solution or by freezing a polymer or like
15 substance having a higher melting point than the solution. (Bevan, C.D., Mutton, I.M., "Use of Freeze-Thaw Flow Management for Controlling and Switching Fluid Flow in Capillary Tubes," 1995, 67 Anal. Chem. 1470-1473).

 Advantageously, the pressure driven fluid transfer system of the present invention has the benefit of performing low-volume, uniform liquid transfer and
20 liquid processing in parallel and is expandable to any number of capillaries. Likewise, the system has the capability of transferring solutions from one arbitrary reservoir configuration to another.

 Briefly stated, there is provided a method and apparatus consisting of at least one capillary, a pressure box having first and second means for aligning the capillaries
25 from one set of wells to a second set of wells, and applied pressure or pressure differential transfers small amounts of liquid uniformly and in parallel. A method of accurately controlling a desired volume of fluid flow is particularly useful for transferring liquids from a microtiter dish to a Capillary Array Electrophoresis Microplate having liquid wells spaced in a radially symmetric configuration.

According to a feature of the invention, there is provided a liquid-handling system for transferring liquid from at least one first container to at least one second container, which comprises: a means for applying pressure to a box containing at least one first container, at least one capillary tube having predetermined length and a predetermined internal diameter, wherein a first end of the predetermined tube is positioned near the bottom of said first container, the predetermined tube sealed through a wall of said box in a pressure-tight manner, and further extending to a predetermined second container and, means for increasing the pressure within the box, such that the liquid contained in the first container is transferred through said capillary tube to the second container when the pressure is raised within the box.

According to another feature of the invention, there is provided a method for using a liquid system for transferring a predetermined amount of said liquid from said first container holding a first volume of said liquid to said second container comprising the steps of calibrating said capillary tube by filling said first container with said liquid, filling said capillary tube with said liquid, increasing said pressure within said box to a predetermined pressure for a predetermined period of time to transfer a quantity of said liquid to said second container, measuring said quantity of said liquid thus transferred with a means for measuring; and, calculating the measured amount of liquid transferred per unit time, calculating the transfer time required to transfer said predetermined amount of liquid, and, increasing the pressure within said box to said predetermined pressure for said transfer time to transfer said predetermined amount of liquid from said first container to said second container.

Likewise, it is contemplated that the present invention encompasses dual vacuum creation means, located at either end of a capillary tube, or an array of the same. Further, it is noted that the instant teachings embrace the transfer of liquid by known, or developed pressure differentials being the driving force behind said transfers and multiple boxes or the like means for containing, including transfers driven by differential gravitational potentials.

Description of the Figures

The above-mentioned and other objects, features, and advantages of this invention and the manner of obtaining them will become more apparent taken in conjunction with the accompanying drawings. These drawings depict only a typical
5 embodiment of the invention and do not therefore limit its scope. They serve to add specificity and detail, in which:

FIG. 1 is a schematic of a microplate loading system according to an embodiment of the present invention;

FIG. 2 is a graphical depiction plotting displaced volume on the y-axis against
10 time on the x-axis where the transfers have been driven by capillary loading systems which are embodiments of the present invention;

FIG. 3 is another schematic showing loading of a common reagent solution into multiple reservoirs according to an embodiment of the present invention;

FIGS. 4A and 4B illustrate liquid capture using a cold plug according to
15 embodiments of the present invention;

FIG. 5 is a schematic depiction of the flow of an air or liquid flow cavity according to embodiments of the present invention whereby a small region of the capillary array shown in Fig. 1 and Fig. 3 is heated or cooled;

FIGS. 6A – 6C illustrate solution removal and loading with a capillary array
20 according to an embodiment of the present invention; and,

FIG. 7 is an illustration showing a method for simultaneous or sequential removal and loading from a capillary array according to an embodiment of the present invention.

Detailed Description of Preferred Embodiments of the Invention

25 Heretofore undisclosed use of pressure differentials for the forward and reverse transfer of fluids through capillaries are disclosed according to the teachings of the present invention. Likewise, those skilled in the art will readily understand the utility of such teachings for use with rapidly evolving sampling technology for DNA and/or like biomolecular species, compounds and/or substituent elements, moieties or-

structures.

The present inventors have discovered that preferred embodiments of the present invention are useful in facilitating the revolution in separation science being effected by rapid and highly parallel electrophoretic analysis. (Simpson, P.C., Roach, D., Woolley, A.T., Thorsen, T., Johnston, R., Sensabaugh, G.F., & Mathies, R.A., 1998, 95 Proc. Natl. Acad. Sci. U.S.A. 2256-2261. Seiler, K., Harrison, D.J. & Manz, A. 1993 65 Anal. Chem., 1481-1488. These references are expressly incorporated within the instant application by reference.).

CAE Microplates (as referenced above in Background of the Invention) are effective for performing extremely rapid electrophoretic separations of nucleic acids such as short tandem repeats ("STR"), single nucleotide polymorphism ("SNP"), restriction fragment length polymorphism ("RFLP") and sequencing analysis, as well as amino acids and other analytes. (Woolley, A.T., Sensabaugh, G.F., & Mathies, R.A., 1997, 69 Anal. Chem. 2181-2186; Woolley, A.T., & Mathies, R.A., 1995, 67 Anal. Chem. 3676-3680; Schmalzing, D., Koutny L., Ziaugra, L. Matsudaira, P. & Ehrlich, D., 1997, 94 Proc. Natl. Acad. Sci. U.S.A. 10273-10278; Schmalzing, D., Koutny L., Ziaugra, L. Matsudaira, P. & Ehrlich, D., 1998, 70 Anal. Chem. 2303-2310.).

Likewise, the rapid pace now conventional under such mechanisms may be performed in time-spans as short as from about thirty seconds to about 2 minutes for fragment sizing, (Woolley, A.T., & Mathies, R.A., 1994, 91 Proc. Natl. Acad. Sci. U.S.A. 11348-11352) and from about 8 to about 20 minutes for sequencing. (Woolley, A.T. & Mathies, R.A., 1995, 67 Anal. Chem. 3676-3680; Schmalzing et. al., 1998, 70 Anal. Chem. 2303-2310.).

Prominent among the challenges of the development of CAE Microplate technology has been the need to load the microplates in a facile manner, that is rapid enough to keep up with the analysis speed of the micro-device. In some designs, the liquid wells on a CAE Microplate are spaced orthogonally on an 8 x 12 array, making them susceptible to use in conjunction with automated robotics. (Simpson, P.C.,

Roach, D., Woolley, A.T., Thorsen, T., Johnston, R., Sensabaugh, G.F., & Mathies, R.A., 1998, 95 Proc. Natl. Acad. Sci. U.S.A. 2256-2261.).

Problematic among robotic systems are the difficulties which arise with respect to mechanical complexity (and failure), pricing schemes, and the slow operation speed typical of such systems. (Watson, A., Smaldon, N., Lucke, R. & Hawkins, T. 1993, 362 Nature [London] 569-570; Hawkins, R.L., McKernan, K.J., Jacobot, L.B., Mackenzie, J.B., Richardson, P.M. & Lander, E.S., 1997, 276 Science 1887-1889; and Buxton, E.C., Westphall, M. Jacobson, W., Tong X. C. et al., 1996, 8 Laboratory Robotics and Automation 339-349.).

Turning now to Fig. 1, the basic format of a pressure loader according to an embodiment of the present invention is shown generally at 101. A first section 103 includes a means for sustaining a pressure gradient between solutions in contact with two ends to drive transport, as shown here as a pressure box assembly, which houses one end of an array of capillaries 107. A first manifold 105 properly spaces the capillaries and a solution to be transferred.

It will become readily apparent to those of skill in the art that alternatives abound for the use as the means for sustaining a pressure gradient between solutions in contact with two ends to drive transport, preferably a pressure box. For example, one could simply place a plate on a microtiter sample dish sealed with o-rings and apply pressure as well. By putting such a dish in a pressure box, a basic embodiment is illustrated, but is not intended to limit the teachings of the present invention, which may be manifested in any number of "boxes" or like containing/pressure gradient housing means.

Fused silica capillary array 107, is comprised of a multiplicity of individual capillaries 120 (or may be only one capillary 120), and makes up the second section of the illustrated embodiment of the present invention. Likewise, a second manifold 109 is effective for receiving capillaries and to space them into any desired spatial orientation, for example for a desired second well, or array of the same. In this illustrative embodiment, a CAE Microplate 111 is shown. Those having a modicum

of skill in the art will readily understand that line 113 connects to a computer controlled pressure source, and that pressure box 103 includes conventional articles such as the illustrated microtiter dish 115.

Pressure box 103 further consists of a chamber in which fluid filled containers
5 or liquid containing plates, such as conventional microtiter plates can be placed. One end of the capillaries extends through the top of the pressure box and are spaced by a manifold in a pattern that matches the layout of the reservoir.

As shown in Fig. 1, fused silica array 107 is illustrative of the instant teachings and those skilled in the art will readily understand how the fluid transfer system of the
10 present invention consists of one or an array of capillaries through which the liquids are transferred. The volume of solution in the capillaries is determined by the inner diameter and the length of the respective capillary.

According to a preferred embodiment of the present invention, such a configuration of the loading system may be in a range of from about 30 cm long
15 capillaries with 75 micron inner diameter and 200 micron outer diameter to an acceptable deviation therefrom. This gives the capillaries an internal volume of approximately 1.325 microliters. The system uses pulled glass capillaries with external polyamide coatings to transfer the liquids; however, any type of capillary or tube with the desired internal volumes can be used, including plastics, or Teflon, such
20 as would be known to those skilled in the art. Thin wall metal or stainless steel capillaries could likewise be used.

Still referring to Fig. 1, the second manifold 109 functions as a capillary spacer, and the main function of this portion of the capillary loading system is to space the capillaries into an array that matches the spacing of the receiving reservoirs.
25 The second manifold 109 is also used to maintain consistent height of the capillary ends to ensure uniform liquid dispensing.

Likewise, according to empirical data derived from preferred embodiments and known information for performance of the present invention operational algorithmic expressions further defining the instant teachings have been adduced by

the present inventors. In sum, the flow characteristics of this system follow in accordance with theoretical calculations of low Reynolds number pipe flow. An equation for expressing such volumetric flow rate (Q), is described by Equation 1:

$$Q = \frac{\pi \Delta p r^4}{8 \mu l} \quad 5 \quad \text{Eq. 1}$$

Where Δp is the differential pressure between the two ends of the capillaries, r is the radius of the capillary, μ is the viscosity of the fluid and L is the length of the capillary. An equation for displaced volume (V) is linear with respect to time (t) and is shown by Equation 2:

$$V = \frac{\pi \Delta p r^4 t}{8 \mu l} \quad \text{Eq. 2}$$

15

Referring now to Figure 2, measurement of de-ionized H₂O displacement versus time for four different applied pressures on a 30 cm long, 75 micron inner diameter capillary is represented graphically. Volumes of water were collected from sets of three capillaries and weighted to calculate the volume and time as well as a linear relationship between the displaced volume and applied pressure, both of which follow theoretical predictions to fall within the expected range appropriate for experimental error. The capillary-to-capillary variance was measured in a similar manner as above. Two sets of data at different pressures (each set consisting of 6 groups of capillaries) were collected and measured, yielding a standard deviation of 3 to 4% of the collected volumes.

Among the inventive features of the present invention is an unprecedented capability for transferring solutions from one reservoir to multiple reservoirs. This loading methodology is likewise used to fill the cathode and waste reservoirs, useful for a variety of applications. For example, CAE microplates have been generated

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which use standard cross injectors on a 4 inch diameter substrate, use a single common anode reservoir thus reducing the needed reservoir count to $3N + 1$, and provide novel enhanced means for electrically addressing chips having from 12 channels up to 96 channels or more.

- 5 Likewise, grouping of channels in different configurations, for example at the anode end, has facilitated a plurality of alternate CAE microplate designs, including those having an ability to be used with a linear confocal scanner. Such embodiments may employ, for example, $50\mu\text{m}$ -wide channels spaced apart $90\mu\text{m}$ for a total array width of 1.1 to 1.2 mm. (Mathies, R.A., Simpson, P.C., & Woolley, A.T., "DNA
10 ANALYSIS WITH CAPILLARY ARRAY ELECTROPHORESIS MICROPLATES," Micro Total Analysis Systems >98, 13-16 October 1998, Proc of the "TAS '98 Workshop, 1-70.).

Referring now to Figure 3, the present invention is effective to fill the cathode and waste reservoirs in the CAE Microplate 111 shown with a common buffer.

- 15 According to this embodiment of the present invention, fused silica capillary array 107, is comprised of a multiplicity of individual capillaries 120 (or may be only one capillary 120), and in the illustrated embodiment is grouped into one reservoir 103 which is the pressure box, at the loading end 115 and laid out in an array corresponding to the cathode and waste reservoirs in the CAE Microplate.

- 20 Pressure is applied to the common loading reservoir 103 and equal amounts of buffer can be transferred to all of the waste reservoirs and/or cathodes in parallel. Fluid level is shown by arrow 117 in pressure box 103, and the line flowing to computer controlled pressure source 113 is likewise illustrated, but not shown.

- Referring now to Figure 4, the present invention further teaches liquid capture
25 using temperature control, including liquid capture using a cold plug as shown in this schematic. Fig. 4A shows a situation according to the present invention where there is fluid flow, and Fig. 4B shows no fluid flow, owing to ice plug 121, lodged in capillary 120. It is noted that the Fig. 4B shows still fluid (not frozen) solution 122, and ice plug 121.

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One of the longstanding challenges to uniform transfer of liquids through the capillaries is in the variability of liquid flow during the initial filling of the capillaries. It is known that such variability could be variously due to differences in the quality of the ends of the capillaries, the condition of the surface of the capillaries and/or blockage in the capillaries.

Once the capillaries are filled, the variability in filling rates decreases to an acceptable variance of about 3 to 4% standard deviation of the loaded volume. To ensure the capillaries are completely filled before dispensing the solution into the receiving reservoirs, a "capture" method can be used to stop the liquid flow near the end of the capillary. This can be accomplished by cooling a small region near the end of the capillary to below the freezing point of the liquid as demonstrated schematically in Figure 4.

When the fluid reaches the cold region 122 defined by the capillary cooler 119, the front end of the solution will freeze and stop the flow of liquid. When all capillaries are filled with the tips frozen at the cold spot, pressure is removed and the temperature can be rapidly elevated to melt the ice plug. Pressure can be reapplied to dispense the fluid. The temperature can be controlled by several methods, including a Peltier cooling/heating system, resistive heating system, cryogenic fluids, flow system or an air flow system.

The air flow system, shown in Figure 5, consists of a narrow air flow cavity 125 which contains a section of the capillary or capillaries 120. A continuous flow of temperature-controlled air passes through the chamber in the direction shown by the arrow at 127 to heat or cool the capillaries. The chamber can also be heated by hot water or cooled by liquid nitrogen, although several other cooling fluids or gases can be used. The chamber walls 129 are well insulated so that the temperature gradient in the capillary 120 is contained primarily to the thickness of the insulator.

The present invention further teaches other liquid stop methods. For example, another method of stopping the flow of the liquids is to use a bolus of a higher melting point fluid that will solidify when it enters the capillary. This can be a polymer or

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wax substance or immiscible inert fluid such as a fluorocarbon that floats on the top of a heated aqueous liquid. When all of the liquid is pressure filled through the capillary, the wax enters the capillary, cools and solidifies, stopping the fluid flow. The temperature of the capillary can also be controlled to allow the polymer through to a specific location within the capillary. Although there are advantages to using the polymer method, such as the ability to transfer liquid with zero dead volume, the frozen liquid plug method is advantageous because polymers may prove difficult to completely remove and can clog the capillary.

Lowering the pressure around the CAE microplate can also effect the primary transfer, and this is noted and dealt with by the instant teachings. Further vacuum cleanup, or transfer in either direction with involved liquid wells is contemplated by the inventors to be both necessary and within the scope of the claimed subject matter of the present invention. This is due to the fact that in some situations it is necessary to remove solutions from the CAE Microplate reservoirs before the new solution can be deposited into the reservoirs.

Referring now to Figure 6, solution removal and loading with a capillary array is shown in three steps [labeled A, B and C for simplicity of illustration]. This schematic diagram demonstrates a method of applying a vacuum to the pressure box 103 (not shown) and sucking out the excess solution from reservoir 131 (A). The excess solution can be expelled from capillary 120 into a waste container located at 133, but not shown in step (B) and the desired solution can be deposited into the vacant liquid holes using the same capillary (C).

Referring now to Figure 7, a two, or more, capillary per reservoir system can be used, for the simultaneous removal and loading from a capillary array. Each capillary 120 shown in Figure 7 is used in accordance with this method, whereby one capillary 120 is used to vacuum remove the undesired liquids and the second 138 is used to deposit the new liquids. Vacuum removal of undesired solution in the direction of waste container 133 (not shown, but direction of travel is indicated by the arrow). Likewise, new solution from the microtiter plate (not shown, but direction of

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travel is indicated by the arrow) travels into second capillary 138 by means of the pressure fill of new solution. Likewise, one could also connect the microplate to three (or any desired number of) different boxes.

- Further, it is understood that the invention includes embodiments where the
- 5 array commencing from the microplate bifurcates and some of the capillaries go to a first pressure box which is used to deliver reagents to the microplate and other capillaries go to a second vacuum chamber that is used to remove fluids from the microplate, and the like arrangements or multiples attachments, appendages or complements such as would be within the scope of the appended claims.
- 10 Having described preferred embodiments of the invention with reference to the accompanying drawings, it is to be understood that the invention is not limited to those precise embodiments, and that various changes and modifications may be effected therein by one of skill in the art without departing from the scope or spirit of the inventions defined in the appended claims.

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WHAT IS CLAIMED IS:

1 1. A liquid-handling system for transferring liquid back and forth from at
2 least one first container to at least one second container, comprising:
3 a means for sustaining a pressure differential between solutions in contact
4 with two ends to drive transport,
5 at least one capillary tube having predetermined length and a predetermined
6 internal diameter, wherein a first end of said predetermined tube is positioned near
7 the bottom of said first container, and extends to a predetermined said second
8 container; and,
9 means for increasing the relative pressure within said means for sustaining
10 a pressure gradient in contact with two ends to drive transport;
11 whereby at least one of said liquid contained in said first container is
12 transferred through said capillary tube to said second container when said pressure
13 gradient or difference is applied.

1 2. The system as defined in claim 1, wherein said predetermined tube is
2 sealed through a wall of said means for sustaining a pressure differential between
3 solutions in contact with two ends to drive transport in a pressure-tight manner,
4 containing said at least one first container.

1 3. The system as defined in claim 1, whereby at least one of said liquid
2 contained in said first container is transferred through said capillary tube by means of at
3 least one of an intrinsic and an extrinsic vacuum source.

1 4. The system as defined in claim 1, further comprising:
2 a first capillary tube spacing means to position a first end of said at least
3 one capillary tube near the bottom of said first container; and,

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4 a second capillary tube spacing means to position a second end of said at
5 least one capillary tube in a manner to deliver said liquid to said second container.

1 5. The system as defined in claim 4, wherein said first capillary tube spacing
2 means is a first manifold and said second capillary tube spacing means is a second
3 manifold.

1 6. The system as defined in claim 5, further comprising a plurality of said
2 first containers deployed in a first array, and a plurality of said second containers
3 deployed in a second array.

1 7. The system as defined in claim 6, further comprising:
2 a first translation subsystem means for transferring said first array in and
3 out of said means for sustaining a pressure gradient between solutions in contact
4 with two ends to drive transport, containing said at least one first container;
5 a second translation subsystem means for raising and lowering said first
6 array;
7 a third translation subsystem means for moving, transferring, raising and
8 lowering said second array; and
9 at least a supplemental means for moving, transferring, raising and
10 lowering a microplate.

1 8. The system as defined in claim 7, further comprising computer means to
2 control said first, second, and third translation means.

1 9. The system as defined in claim 1, wherein said at least one capillary tube
2 is constructed of a material selected from the group consisting of pulled glass, pulled
3 glass with an external coating, polyamide, polyethylene, polypropylene,

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4 polytetrafluoroethylene, polyester, PEEK(polyethylenetherketone), stainless steel and
5 other chemically unreactive materials.

1 10. The system as in defined claim 9, wherein said means for raising said
2 pressure within said means for sustaining a pressure gradient between solutions in contact
3 with two ends to drive transport comprises a source of pressurized gas selected from the
4 group consisting of air, nitrogen, argon, helium, combinations of the same and the like.

1 11. The system as defined in claim 10, wherein said pressure is at least one of
2 raised to between about 0.5 lb. per square inch and about 10 lb. per square inch and
3 drawn by a vacuum source having a predetermined force value.

1 12. The system as defined in claim 10, wherein said pressure is used with
2 viscous fluids and is within a range of up to about 1000 psi.

1 13. The system as defined in claim 1, wherein said at least one capillary tube
2 has a predetermined length selected from a range of about 10-100 cm. and has a
3 predetermined inner diameter selected from a range of about 10-500 μ m.

1 14. The system as defined in claim 1, whereby solutions are deposited and
2 removed in either direction by at least one of sequential and parallel transport of said
3 solutions from a well having at least two capillaries, including the deposit of two or more
4 solutions to be mixed and removal of a resulting mixture by an additional capillary.

1 15. A method for transferring a predetermined amount of liquid to and from a
2 first container holding a first volume of liquid to a second container and back comprising
3 the steps of:

4 providing a means for sustaining a pressure gradient between solutions in
5 contact with two ends to drive transport;

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6 at least one capillary tube having predetermined length and a
7 predetermined internal diameter, wherein a first end of said predetermined tube is
8 positioned near the bottom of said first container, and extends to a predetermined
9 said second container; and

10 means for increasing the pressure differential between the two
11 ends, whereby said liquid contained in said first container is transferred through
12 said capillary tube to said second container when at least one of said pressure
13 gradient or difference is applied;

14 (a) calibrating said capillary tube;

15 (b) calculating the transfer time required to transfer said
16 predetermined amount of liquid; and

17 (c) increasing the pressure within said box to said
18 predetermined pressure for said transfer time to transfer
19 said predetermined amount of liquid from said first
20 container to said second container when said pressure
21 gradient or difference is applied.

1 16. A method according to claim 15, wherein said means for sustaining a
2 pressure gradient between solutions in contact with two ends to drive transport is
3 preferably a pressure box for containing said at least one first container, and said
4 calibrating step further comprises the steps of:

- 5 • filling said first container with said liquid;
- 6 • filling said capillary tube with said liquid;
- 7 • increasing said pressure within said box to a predetermined
8 pressure for a predetermined period of time to transfer a quantity of
9 said liquid to said second container;

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- 10 • measuring said quantity of said liquid thus transferred with a
- 11 means for measuring; and,
- 12 • calculating the measured amount of liquid transferred per unit time

1 17. A method according to claim 16, wherein said predetermined tube is
2 sealed through a wall of said box in a pressure-tight manner.

1 18. A method according to claim 16, whereby a pressure differential is
2 effected by means of at least one of an intrinsic and an extrinsic vacuum source.

1 19. A method for using the system of claim 14 for transferring a defined
2 amount of liquid from said first container holding a first volume of liquid to said second
3 container comprising the steps of:

- 4 (a) calibrating said capillary tube;
- 5 (b) using said system to deliver defined amounts by:
 - 6 • filling said first container with liquid;
 - 7 • cooling with means for cooling said second end of said capillary
 - 8 tube to below the freezing point of said liquid;
 - 9 • increasing the pressure within said means for sustaining a pressure
 - 10 differential between solutions in contact with two ends to drive
 - 11 transport, whereby liquid fills said capillary tube and forms a
 - 12 frozen plug upon reaching said second end;
 - 13 • thawing said frozen plug with heating means; and
 - 14 • increasing the pressure within said means for sustaining a pressure
 - 15 differential across said tube whereby liquid is expelled from said
 - 16 capillary tube and delivered to said second container.

- 1 20. The method of claim 19, said calibrating step further comprises:
- 2 • filling said first container with liquid;
- 3 • cooling with means for cooling said second end of said capillary
- 4 tube to below the freezing point of said liquid;
- 5 • increasing the pressure differential across said tube, whereby liquid
- 6 fills said capillary tube and forms a frozen plug upon reaching said
- 7 second end;
- 8 • thawing said frozen plug with heating means;
- 9 • increasing the pressure within said whereby liquid is expelled from
- 10 said capillary tube; and,
- 11 • determining said defined amount by measuring with a measuring
- 12 means the liquid thus expelled;

- 1 21. The method as in claim 14 wherein said cooling means is a member
- 2 selected from the group consisting of Peltier cooling systems, cryogenic fluid flow
- 3 systems, liquid nitrogen, liquid air, liquid helium, chilled gases, ice, and solid carbon
- 4 dioxide; and wherein said heating means are a member selected from the group consisting
- 5 of Peltier heating systems, resistive heating systems, air flow systems, and hot water.

- 1 22. A method for stopping and starting the flow of a liquid in the capillary
- 2 tube of the system of claim 1, said method comprising:
- 3 (a) floating, on the surface of said liquid in said first container, a
- 4 chemically inert liquid substance having a freezing point above the
- 5 freezing point of said liquid in said first container and a density
- 6 below the density of said liquid in said first container;
- 7 (b) cooling with means for cooling at least one of said first and second
- 8 end of said capillary tube to below the freezing point of said
- 9 chemically inert substance;

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10 (c) increasing the pressure differential across the tube whereby liquid
11 fills said capillary tube, and flows through until said inert
12 substance forms a frozen plug upon reaching said second end.

1 23. The method as defined in claim 15, whereby a fixed closed volume is
2 defined through the definition or two spatially separate frozen zones, which fixed closed
3 volume may be subject to other reactions.

1 24. The method as defined in claim 14, wherein said means for measuring are
2 at least one means selected from a means for measuring volume and a means for
3 measuring weight.

1 25. The method as defined in claim 15, wherein said means for measuring are
2 at least one means selected from a means for measuring volume and a means for
3 measuring weight.

1 26. The method as in claim 15 wherein said cooling means is a member
2 selected from the group consisting of Peltier cooling systems, cryogenic fluid flow
3 systems, liquid nitrogen, liquid air, liquid helium, ice, and solid carbon dioxide; and
4 wherein said heating means are a member selected from the group consisting of Peltier
5 heating systems, resistive heating systems, gas flow systems, and hot water.

1 27. The method as in claim 14 wherein said substance is a member selected
2 from the group consisting of waxes, polymers and fluorocarbons.

1 28. A method for using the system as defined in claim 14, wherein said system
2 further comprises a waste container, whereby said liquid that has been transferred to said
3 second container is transferred to said waste container, comprising the steps of:

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- 4 (a) positioning said second end of said capillary to the bottom of said
 5 second container;
 6 (b) decreasing said atmospheric pressure within said box to transfer
 7 said liquid from said second container to said capillary;
 8 (c) positioning said second end of said capillary over said waste
 9 container;
 10 (d) increasing said atmospheric pressure within said box to transfer
 11 said liquid in said capillary to said waste container.

1 29. A method as defined in claim 26, wherein a volumetric flow rate (Q) is exercised
 2 in at least one of a forward and reverse direction as described by Equation 1:

$$Q = \frac{\pi \Delta p r^4}{8 \mu L}$$

Eq. 1

6 Where Δp is the differential pressure between the two ends of the capillaries, r is the
 7 radius of the capillary, μ is the viscosity of the fluid and L is the length of the capillary;
 8 and the displaced volume (V) is linear with respect to time (t) and is shown by
 9 Equation 2:

$$V = \frac{\pi \Delta p r^4 t}{8 \mu L}$$

Eq. 2

-22-

30. The method as defined in claim 19, said cooling step further comprising a controlling freezing location located between the first and second ends of said capillary.

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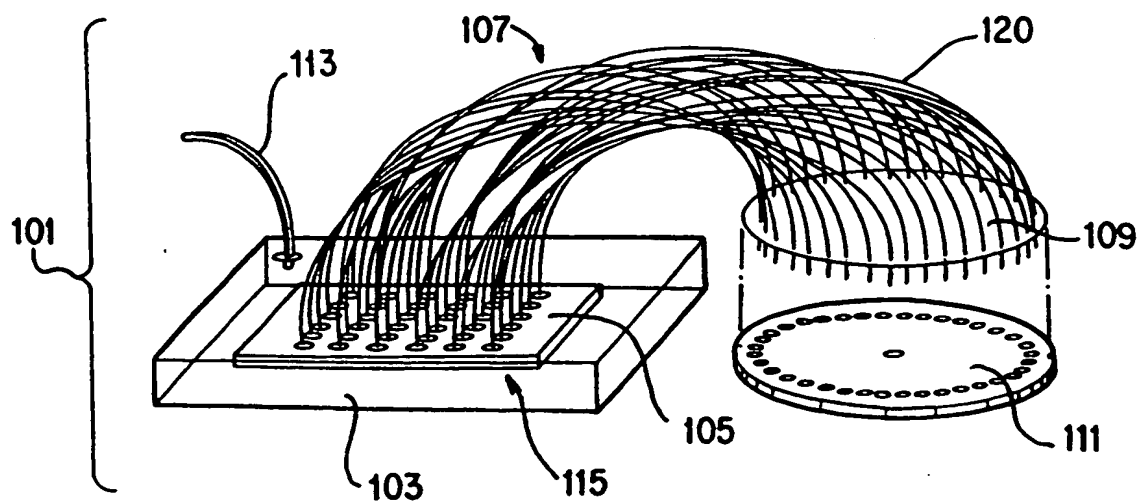


FIG. 1

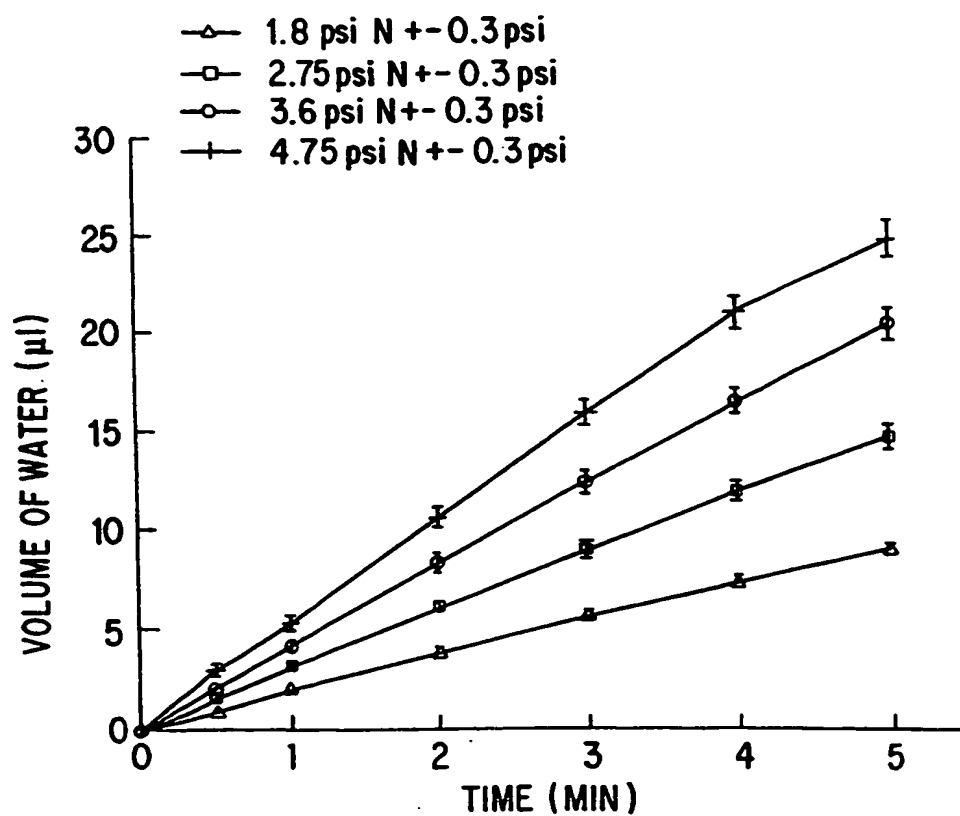


FIG. 2

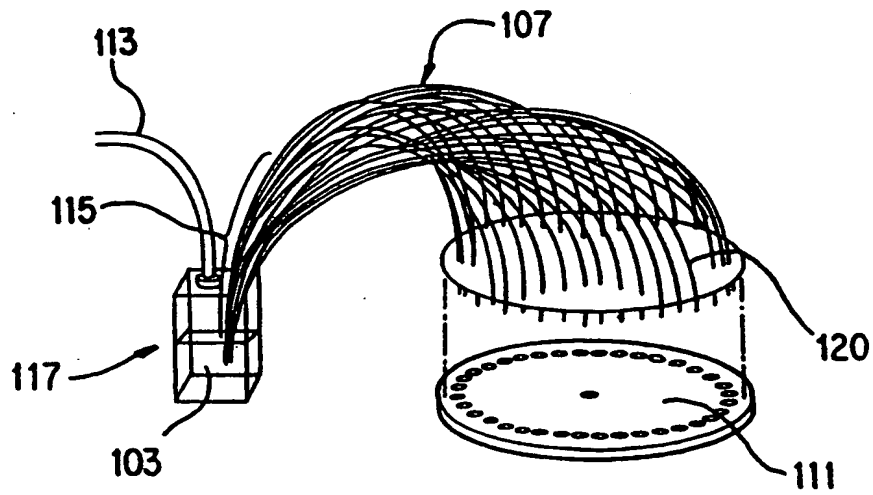


FIG. 3

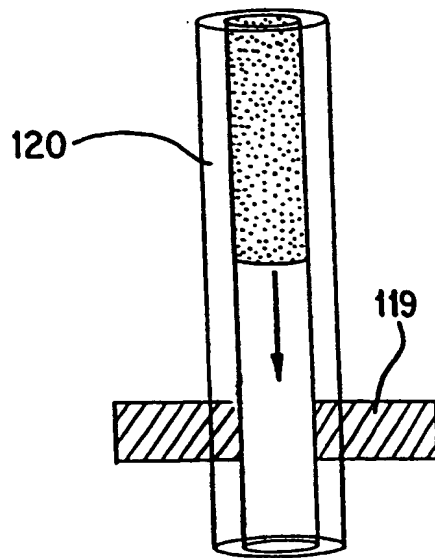


FIG. 4A

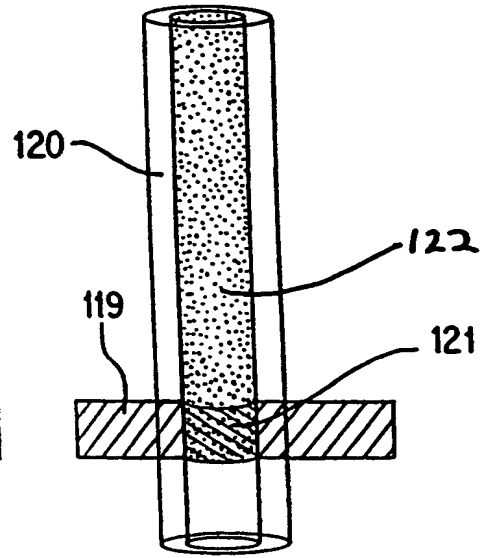


FIG. 4B

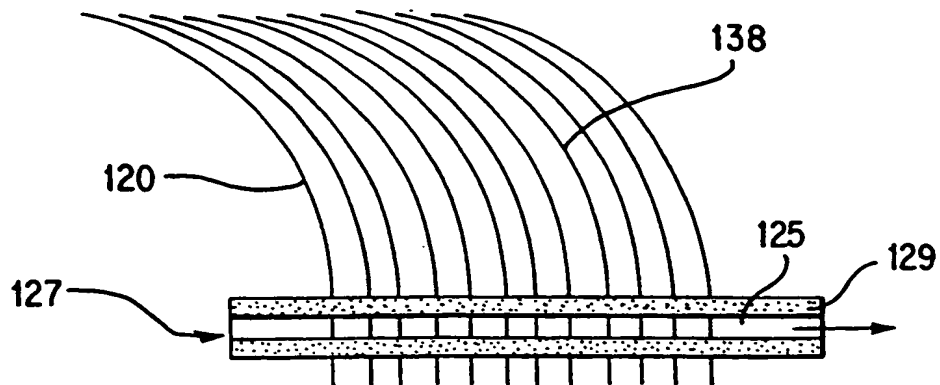


FIG. 5

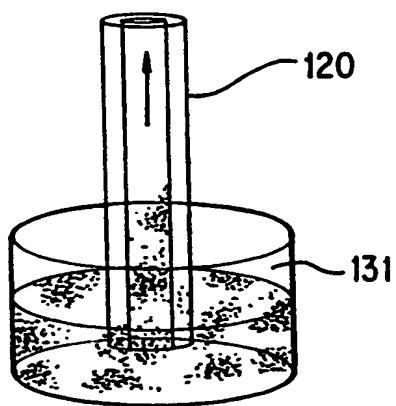


FIG. 6A

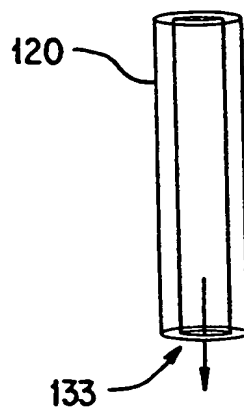


FIG. 6B

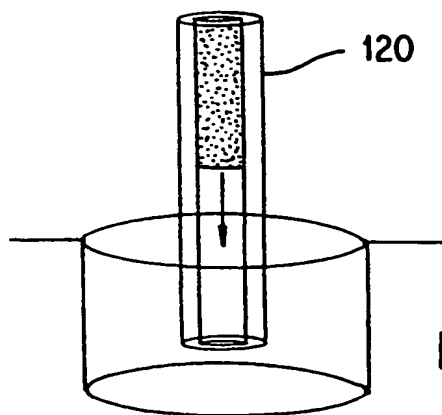


FIG. 6C

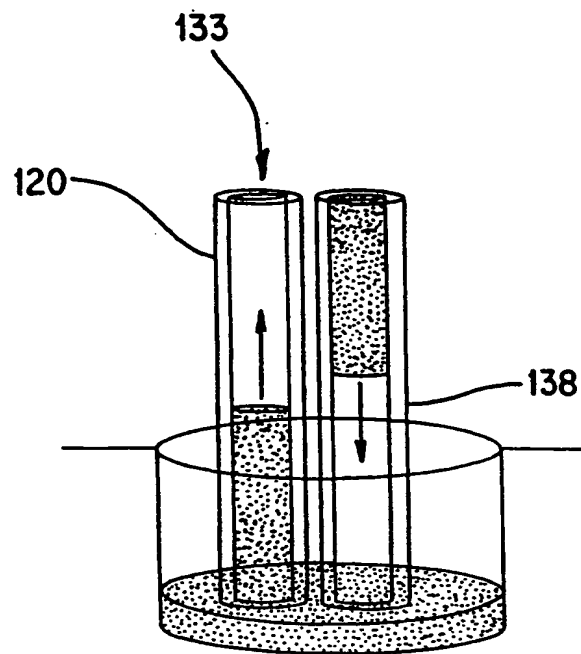


FIG. 7

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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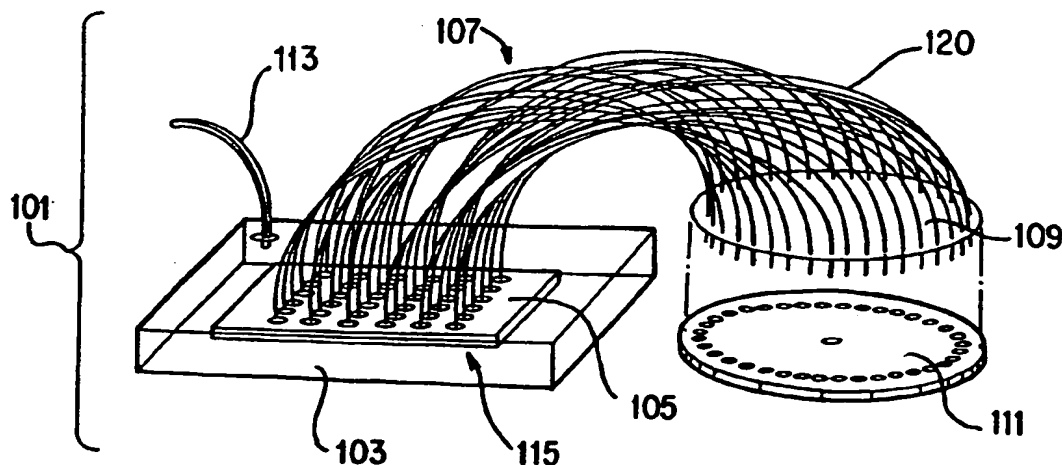
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WO 00/030751 A3

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- (74) Agents: ZAITLEN, Richard, H. et al.; Pillsbury Madison & Sutro LLP, Suite 1200, 725 S. Figueroa Street, Los Angeles, CA 90017 (US).
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3 January 2003
- (72) Inventors: MATHIES, Richard, A.; University of California, Berkeley, Dept. of Chemistry, Berkeley, CA 94720 (US). SIMPSON, Peter, C.; University of California, Berkeley, Dept. of Chemistry, Berkeley, CA 94720 (US).
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MICROPLATE SAMPLE AND REAGENT LOADING SYSTEM



(57) Abstract: Apparatus for forward and reverse transfer of fluids through capillaries, consisting of at least one capillary, a pressure generating or housing means, which preferably includes a box, having first and second means for aligning the capillaries from one set of wells to a second set of wells, and applied pressure differential transfers small amounts of liquid uniformly and in parallel. A method of accurately controlling a desired volume of fluid flow is particularly useful for transferring liquids to and from a microtiter dish to a Capillary Array Electrophoresis Microplate having liquid wells spaced in a radially symmetric configuration, or for maximizing desired transfer of the like or improved novel enhanced patterned arrays.

WO 00/030751 A3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/28014

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 B01L11/00 F16K13/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 B01L G01N B01F F16K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 97 34138 A (MARDIS ELAINE R ;UNIV WASHINGTON (US); PANUSSIS DIMITRIOS A (US);) 18 September 1997 (1997-09-18)</p> <p>page 1, line 8 - line 10 page 3, line 6 - line 7 page 3, line 21 - line 34 page 4, line 9 - line 26 page 5, line 29 - line 36 page 6, line 6 - line 24 page 7, line 32 - line 35 page 8, line 22 - line 23 page 9, line 27 - line 32 page 10, line 2 - line 30 page 12, line 29 - line 30 page 13, line 35 -page 14, line 6</p> <p>--- -/-</p>	1-13

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

16 July 2002

Date of mailing of the international search report

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Name and mailing address of the ISA

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Wyplosz, N

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/28014

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 29736 A (GENOMETRIX INC) 9 July 1998 (1998-07-09) page 22, line 11 -page 23, line 15 page 27, line 6 - line 7 page 28, line 10 - line 13 claims 1,9,12,13,15,17 figures 4,4A,4B ---	1-13
X	EP 0 475 533 B (LAUER LABS B V) 5 February 1997 (1997-02-05)	15-20,28
Y	column 2, line 5 - line 11 column 2, line 38 - line 52 column 3, line 27 - line 46 column 4, line 26 - line 30 column 7, line 52 - line 53 column 9, line 22 - line 25 column 10, line 47 - line 57 column 11, line 16 - line 33 figures 1,6 claim 6 ---	30
Y	FR 2 730 544 A (BIO MERIEUX) 14 August 1996 (1996-08-14) figures 1,2 page 7, line 20 -page 8, line 2 page 6, line 4 - line 20 -----	30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/28014

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 21, 23-26, 29
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20, 28, 30

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box 1.2

Claims Nos.: 21, 23-26, 29

Dependent method claims 21, 24 and 27 are incorrectly written as they depend on system claim 14. Claim 27 relates to a "substance". It has been considered as dependent on claim 22 which is the only claim referring to a "substance". Claim 21 relates to a cooling means and claim 24 to a measuring means. As there are several method claims relating to a cooling means or a measuring means, no obvious correction is possible, and lack of clarity within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

Similarly claims 23, 25 and 26 incorrectly depend on claim 15. Claim 23 and 26 relate to a cooling means and claim 25 relates to a measuring means. However method claim 15 does not mention cooling means nor measuring means. As there are several method claims relating to a cooling means or a measuring means, no obvious correction is possible, and lack of clarity within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-20, 28,30

transfer a predetermined amount of liquid between two containers

2. Claims: 22, 27 (considered as dependent on 22)

method to stop the flow of a liquid in a capillary with a frozen plug

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/28014

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9734138 A	18-09-1997	US 5849598 A	15-12-1998
		AU 2325297 A	01-10-1997
WO 9829736 A	09-07-1998	AU 6646398 A	31-07-1998
		EP 1249705 A	16-10-2002
		EP 0990142 A	05-04-2000
		JP 2001510339 T	31-07-2001
		US 6083763 A	04-07-2000
		US 6331441 B	18-12-2001
		US 6312960 B	06-11-2001
EP 0475533 B	18-03-1992	DE 69124556 D	20-03-1997
		DE 69124556 T	11-09-1997
		EP 0475533 A	18-03-1992
		US 5217590 A	08-06-1993
FR 2730544 A	14-08-1996	AT 184082 T	15-09-1999
		CA 2169403 A	14-08-1996
		DE 69603999 D	07-10-1999
		DE 69603999 T	23-12-1999
		EP 0731303 A	11-09-1996
		FR 2730545 A	14-08-1996
		JP 8297076 A	12-11-1996
		US 5988197 A	23-11-1999

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